209720 "820520DT

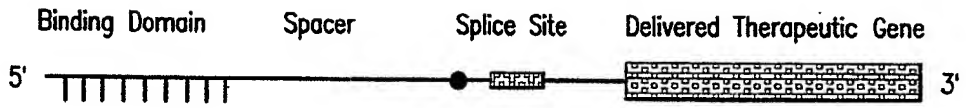


FIG.1A

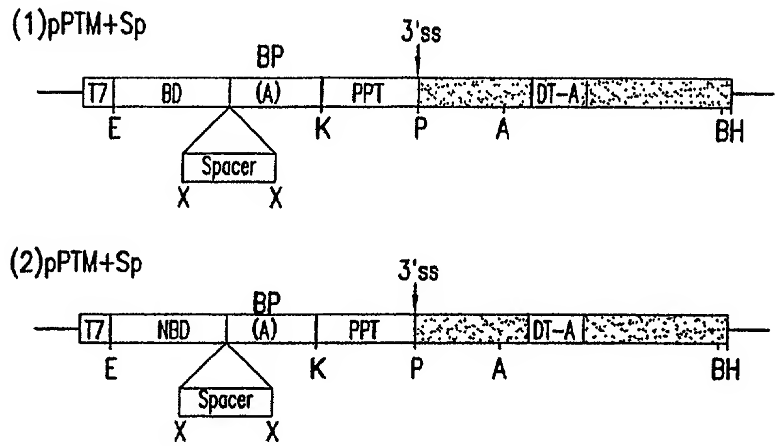

(1)pPTM+Sp
3'ss
BP
T7
BD
(A)
PPT
DT-A
E
K
P
A
BH
Spacer
X
X
(2)pPTM+Sp
3'ss
BP
T7
NBD
(A)
PPT
DT-A
E
K
P
A
BH
Spacer
X
X

FIG.1B

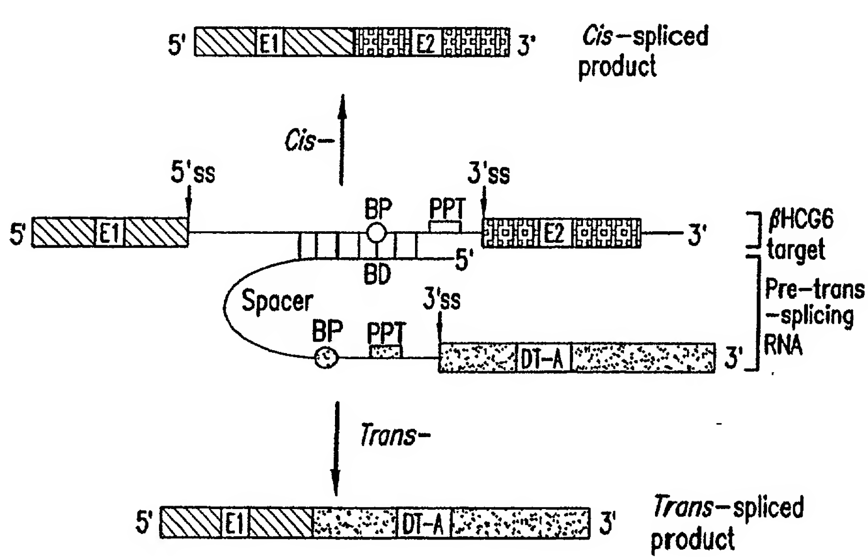

5'
E1
E2
3'
Cis-spliced product
Cis-
5'ss
3'ss
BP
PPT
5'
E1
E2
3'
βHCG6 target
5'
BD
Spacer
3'ss
BP
PPT
DT-A
3'
Pre-trans -splicing RNA
Trans-
5'
E1
DT-A
3'
Trans-spliced product

FIG.1C

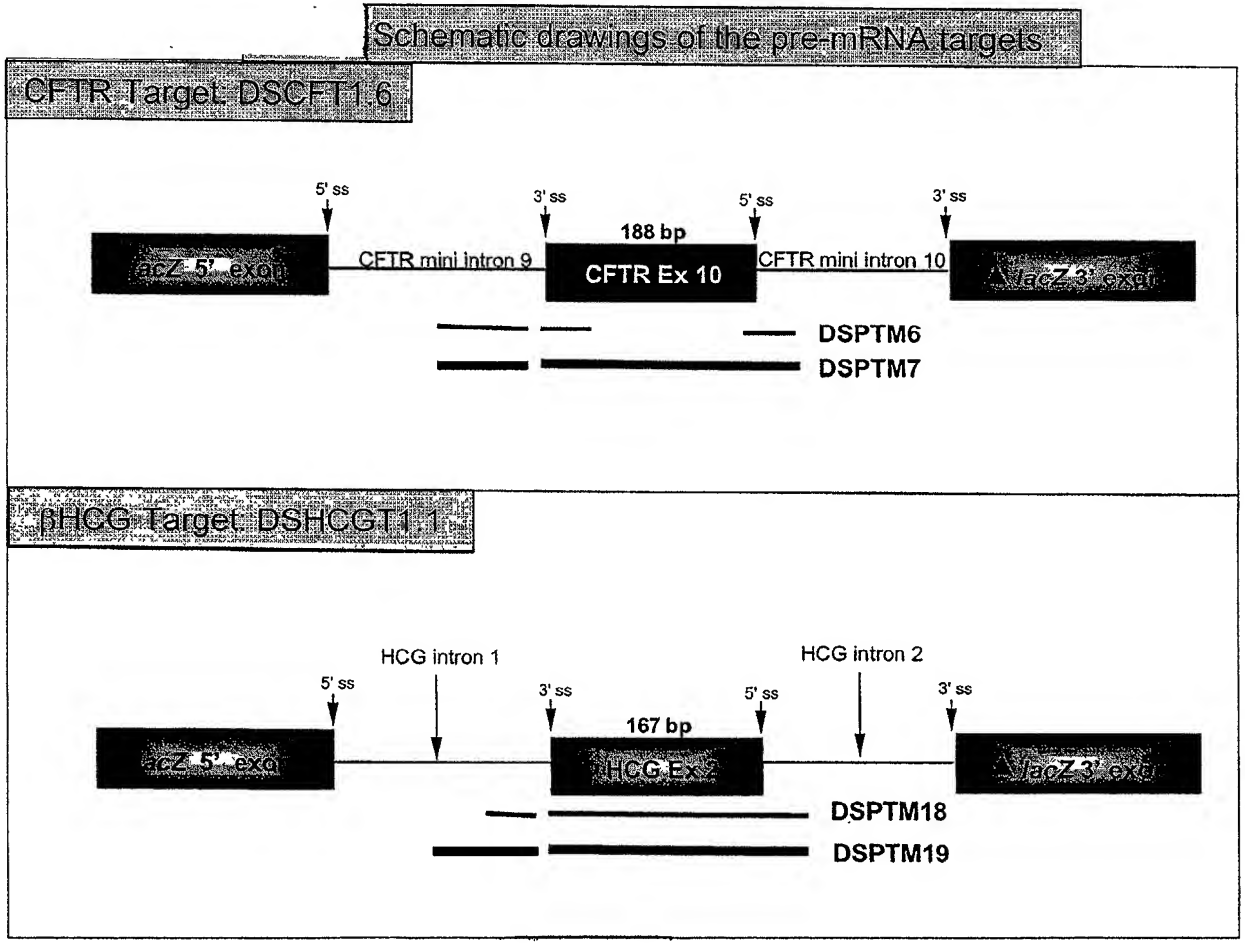

Schematic drawings of the pre-mRNA targets
CFTR Target: DSCFT1.6
5' ss
3' ss
5' ss
3' ss
188 bp
lacZ 5' exon
CFTR mini intron 9
CFTR Ex 10
CFTR mini intron 10
lacZ 3' exon
DSPTM6
DSPTM7
βHCG Target: DSHCGT1.1
HCG intron 1
HCG intron 2
5' ss
3' ss
5' ss
3' ss
167 bp
lacZ 5' exon
HCG Ex 2
lacZ 3' exon
DSPTM18
DSPTM19

Figure 2

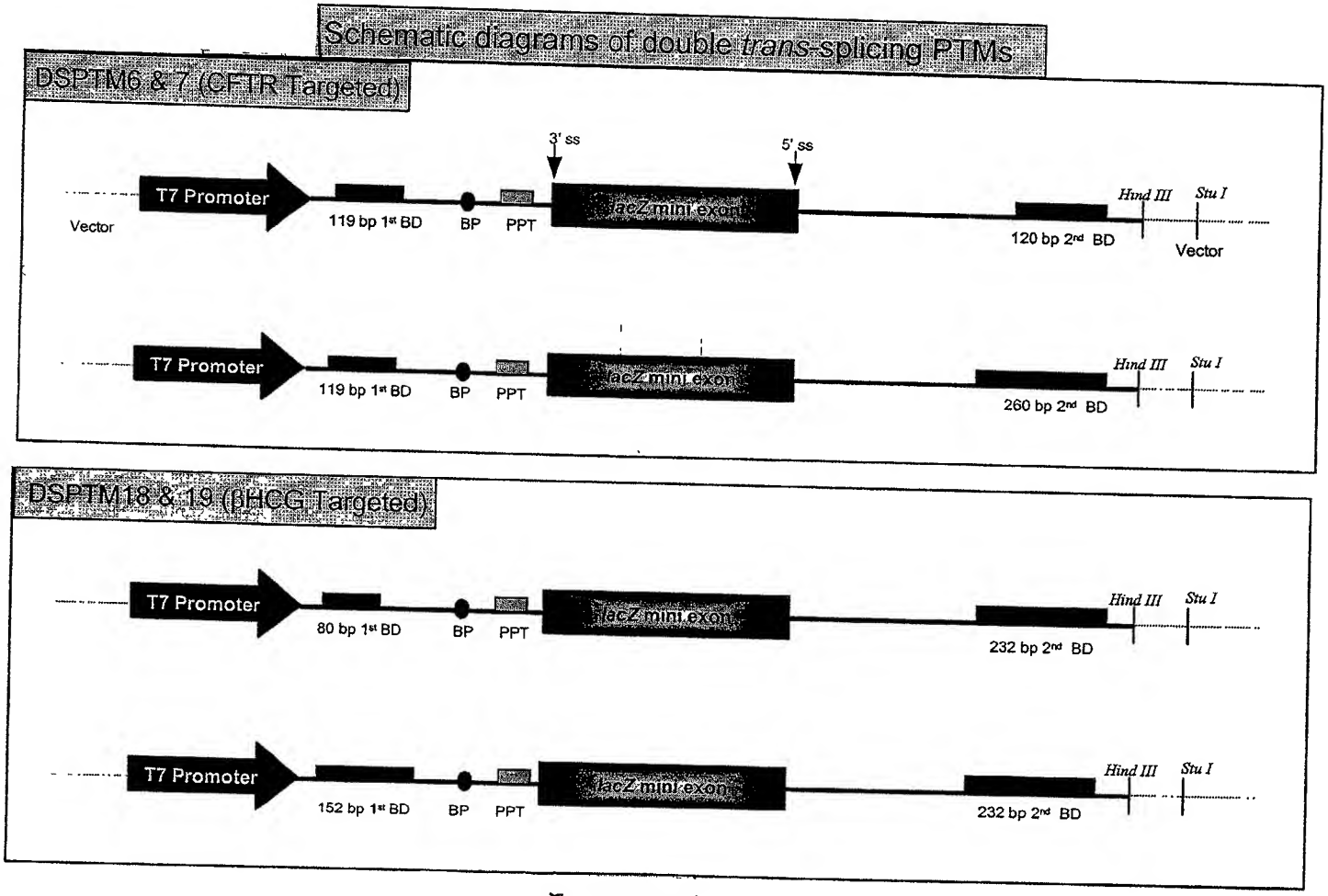
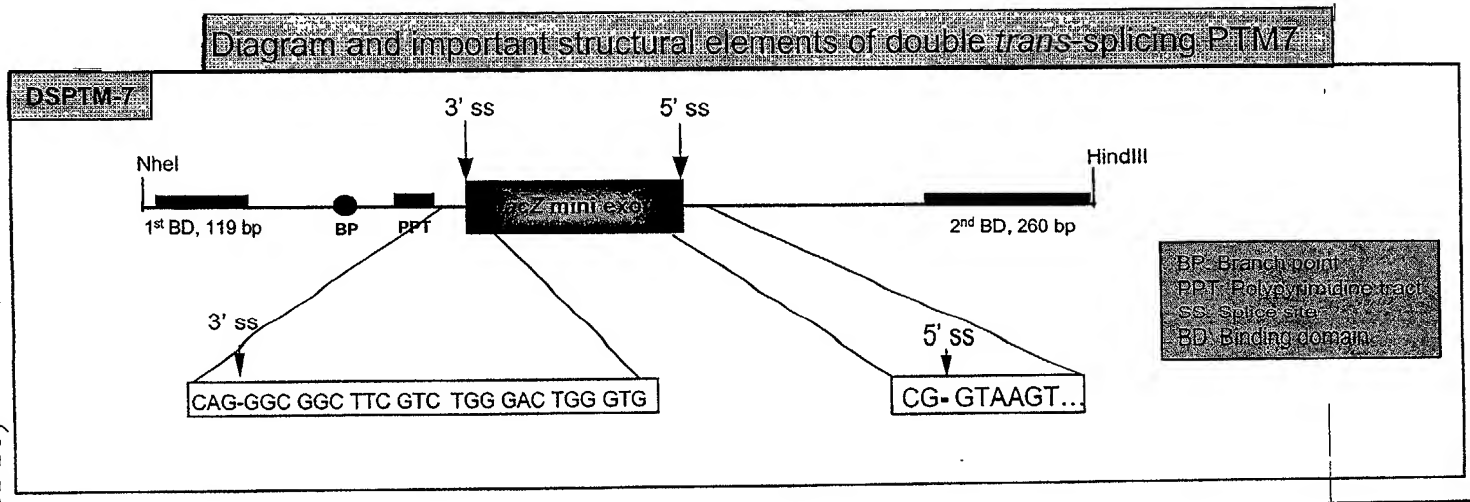

Schematic diagrams of double *trans*-splicing PTMs
DSPTM6 & 7 (CFTR Targeted)
3' ss
5' ss
T7 Promoter
Vector
119 bp 1st BD
BP
PPT
lacZ mini exon
120 bp 2nd BD
Hind III
Stu I
Vector
T7 Promoter
119 bp 1st BD
BP
PPT
lacZ mini exon
260 bp 2nd BD
Hind III
Stu I
DSPTM18 & 19 (βHCG Targeted)
T7 Promoter
80 bp 1st BD
BP
PPT
lacZ mini exon
232 bp 2nd BD
Hind III
Stu I
T7 Promoter
152 bp 1st BD
BP
PPT
lacZ mini exon
232 bp 2nd BD
Hind III
Stu I

Figure 3



1st BD (119 bp) : GATTCACTTGCTCCAATTATCATCCTAAGCAGAAGTGTATATTCTTATTTGTAAAGATTCTATTAACTCATTTGATTCAAAATA TTTAAAATACTTCCTGTTTCATACTCTGCTATGCAC

Spacer sequences: AACATTATTATAACGTTGCTCGAA

BP, PPT and acceptor splice site: TACTAAC T *GGTACC* TCTTCTTTTTTTTTT *GATATC* CTGCAG GGC GGC TTC GTC TGG GAC TGG

BP PPT 3' ss *lacZ* mini exon

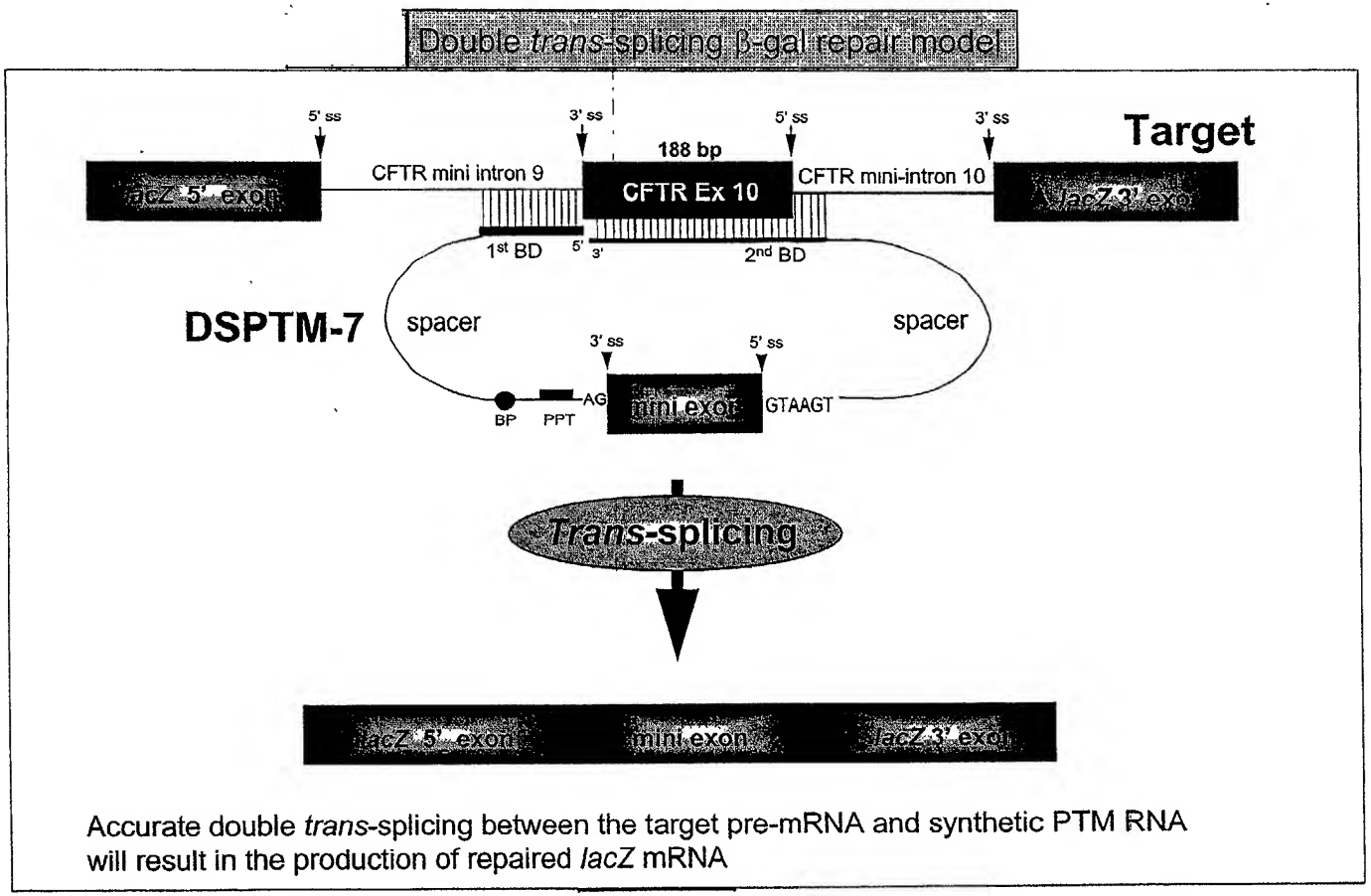
5' donor site and 2nd spacer sequence: TGA ACG *GTAAGT* GTTATCACCGATATGTGTCTAACCTGATTCGGGCCTTCGATACGCTAA GATCCACCGG

lacZ mini exon 5' ss

2nd BD (260 bp): TCAAAAAGTTTTCACATAATTTCTTACCTCTTCTT*GAATTC*ATGCTTTGATGACGCTTCTGTATCTATATTCATCATTGGAA ACACCAATGATTTTTCTTTAATGGTGCCTGGCATAATCCTGGAAAACTGATAACACAATGAAATTCTTCCACTGTGCTTAA AAAAACCCTCT*GAATTC*TCCATTTCTCCCATAATCATCATTACAACTGAACTCTGGAAATAAAACCCATCATTATTAACTCA TTATCAAATCACGC

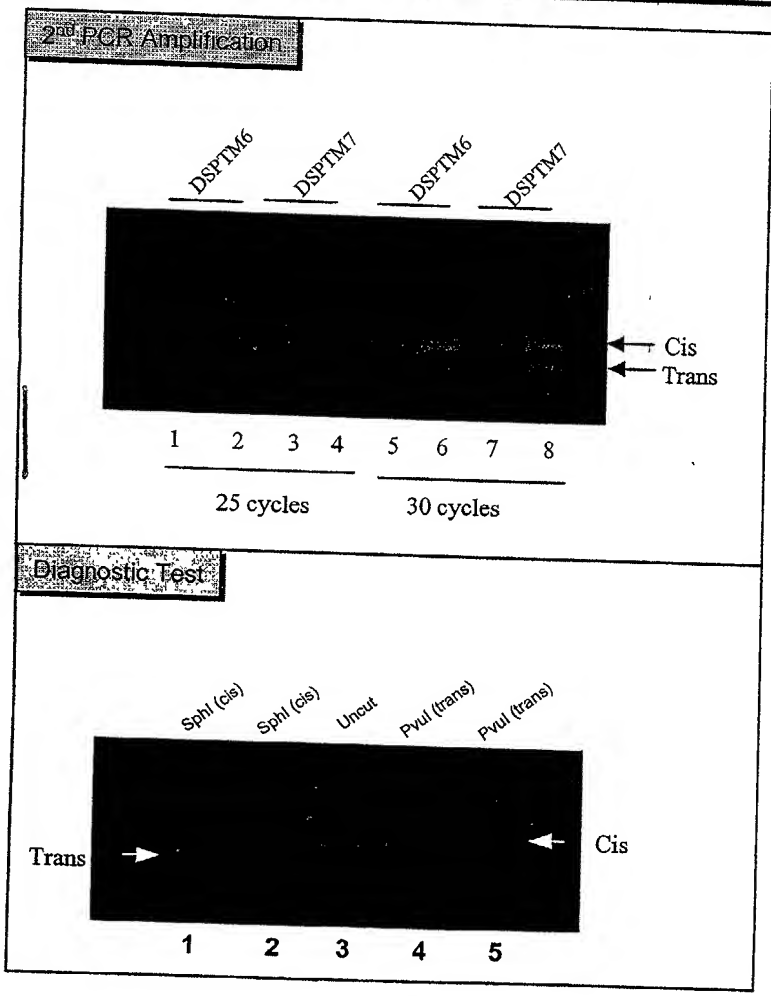
Figure 4

Figure 5



Accurate double *trans*-splicing between the target pre-mRNA and synthetic PTM RNA will result in the production of repaired *lacZ* mRNA

Proof-of-principle of SMaRT using synthetic double splicing PTM RNA in 293T cells



DSPTM6 and 7 (CFTR targeted)

Methods

Transfect 293T cells with DSPTM6 and DSPTM7 *in vitro* transcribed, gel purified RNA (2.5-5.0 µg)

Isolate total RNA, cDNA synthesis (Lac6R), PCR amplification (20 cycls, KI-1F + Lac6R), digest with *Sph* I + *Dde* I (*cis*-specific) at 37°C/ON

Purify double *trans*-spliced product using Biotin-Lac21R probe

PCR amplify the captured *trans*-spliced product (KI-2F+Lac6R). Expected products: *cis*- 260bp; *trans*- 220 bp.

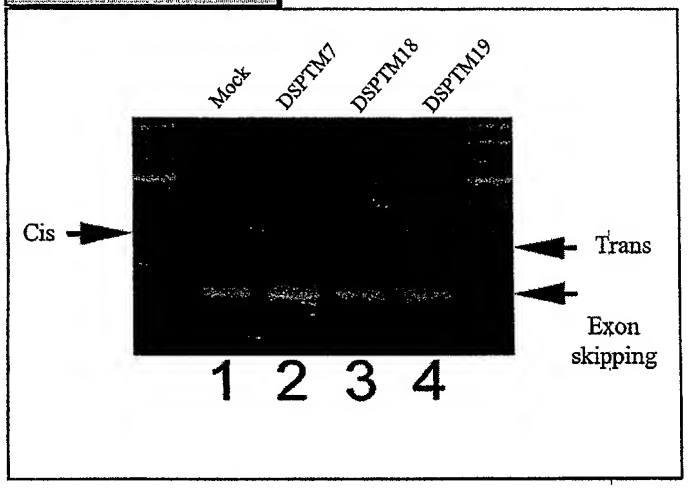
Diagnostic test: Digest PCR product with *Pvu* I (*trans*-specific) and with *Sph* I (*cis*-specific) at 37°C for 2-3 hr

Sequence to confirm the accuracy of double *trans*-splicing

Figure 6A

Proof-of-principle of SMaRT using synthetic double splicing PTM RNA in stable cells

2nd PCR Amplification



DSPTM18 and 19 (HCG targeted)

Methods

Transfect DSHCGT1.1 stable cells with DSPTM7, DSPTM18 and DSPTM19 *in vitro* transcribed, gel purified RNA (2.5-5.0 µg)

Isolate total RNA, cDNA synthesis (Lac6R), PCR amplification (20 cycls, KI-1F + Lac6R), digest with *Sph* I + *Dde* I (*cis*-specific) at 37°C/ON

Purify double *trans*-spliced product using Biotin-Lac21R probe

PCR amplify the captured *trans*-spliced product (KI-2F + Lac6R). Expected products: *cis*- 260bp; *trans*- 220 bp

Sequence to confirm the accuracy of double *trans*-splicing

Figure 6B

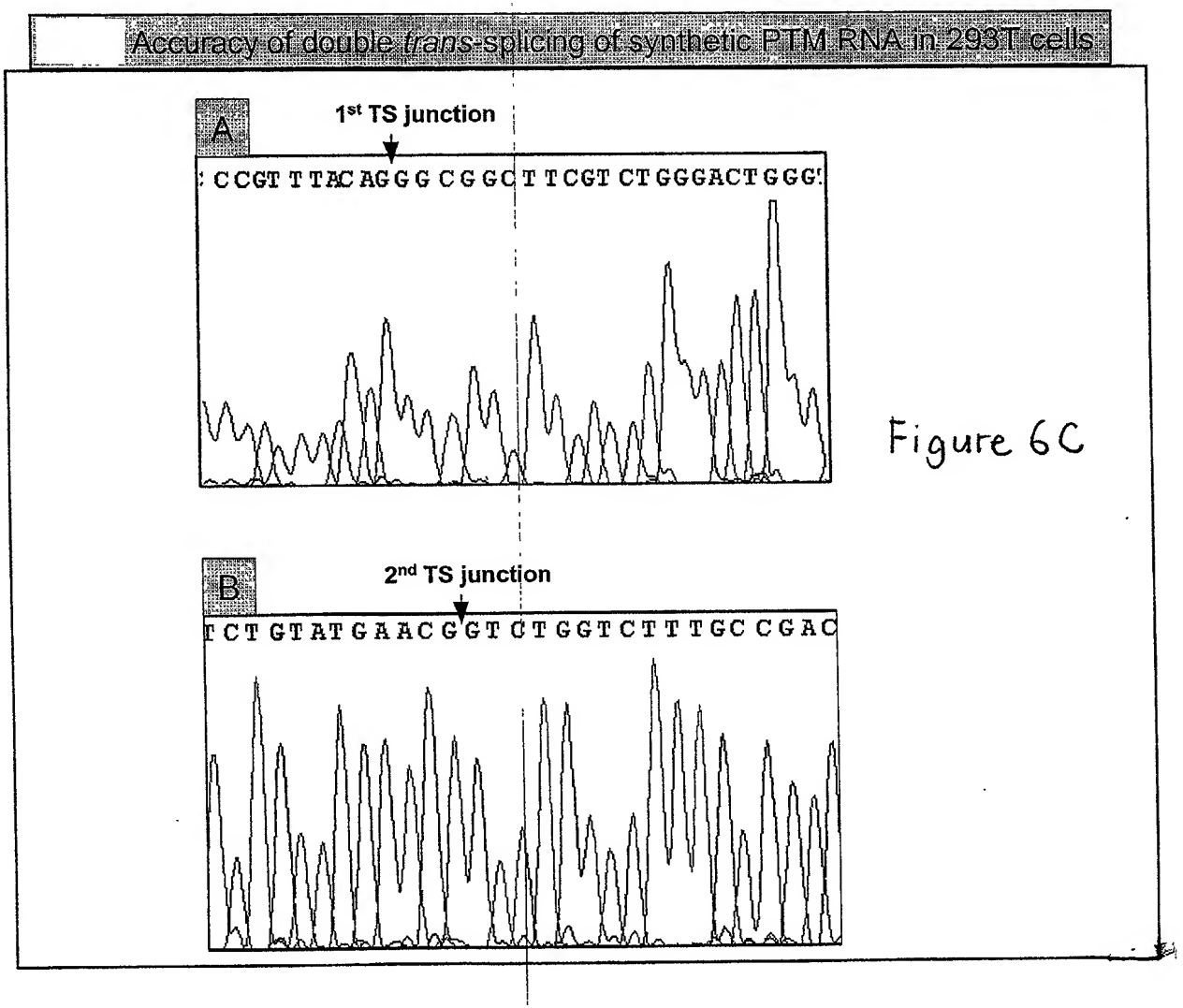

Accuracy of double *trans*-splicing of synthetic PTM RNA in 293T cells
A
1st TS junction
CCGT T TAC AGG G C G GC T T CGT CT GGGACT GGG
B
2nd TS junction
T CT GT AT GA A C G GT C T GGT C T T T GC C GA C

Figure 6C

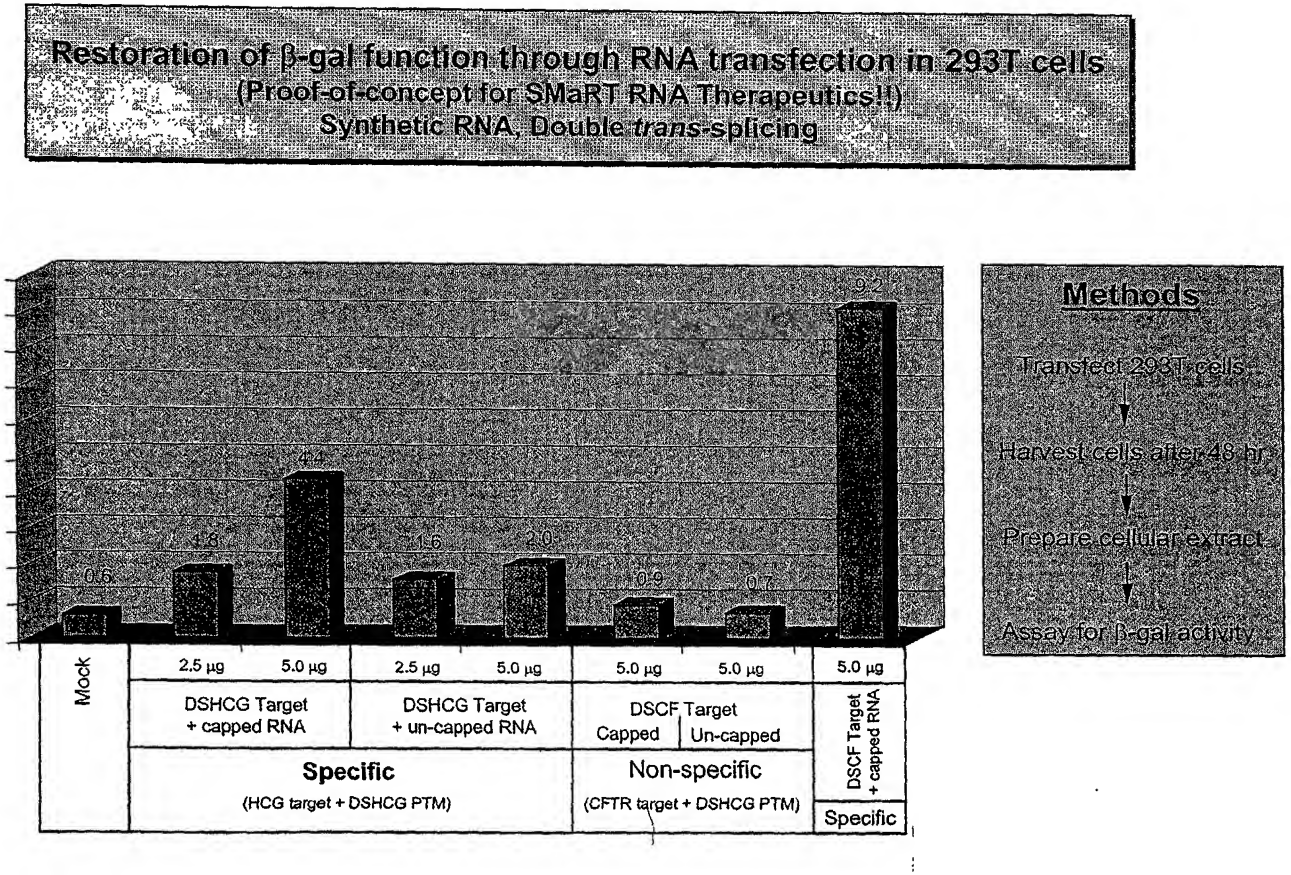

Restoration of β-gal function through RNA transfection in 293T cells
(Proof-of-concept for SMaRT RNA Therapeutics!!)
Synthetic RNA, Double trans-splicing
β-gal Activity (Units/mg of protein)
10
8
6
4
2
0
0.6
1.8
4.4
1.6
2.0
0.9
0.7
9.2
Mock
2.5 µg
5.0 µg
2.5 µg
5.0 µg
5.0 µg
5.0 µg
5.0 µg
DSHCG Target + capped RNA
DSHCG Target + un-capped RNA
DSCF Target
Capped
Un-capped
DSCF Target + capped RNA
Specific
(HCG target + DSHCG PTM)
Non-specific
(CFTR target + DSHCG PTM)
Specific
Methods
Transfect 293T cells
Harvest cells after 48 hr
Prepare cellular extract
Assay for β-gal activity

Figure 7